

Expression and Functional Characterisation of Variola and Monkeypox Virus Tumour Necrosis Factor Receptor (TNFR) Proteins

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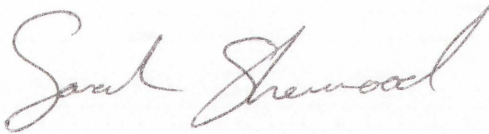
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CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirement for a degree except as fully acknowledged within the text.

I also certify that this thesis has been written by me. Any help that I have received in my research work and in preparation of this thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

A handwritten signature in cursive script, reading "Sarah Sherwood". The signature is written in dark ink and is positioned above the printed name.

Sarah Sherwood

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Inhibiting Tumor Necrosis Factor Receptor Mediated Inflammation. (Oral Presentation)

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LIST OF ABBREVIATIONS

| Abbreviation | Full name |
|--------------------|--|
| ActD | Actinomycin D |
| ANT | Adenine nucleotide translocator |
| Apaf-1 | Apoptotic protease activity factor 1 |
| APRIL | A proliferating-inducing ligand |
| ASFV | African swine virus |
| BAFF | B cell activating factor |
| BCIP | 5-bromo-4-chloro-3-indolylphosphate |
| BID | Bcl-2 interacting protein |
| bp | Base pair |
| BSA | Bovine serum albumin |
| CDC | Centers for Disease Control and Prevention |
| cDNA | Complementary DNA |
| CFP | Cyan fluorescent protein |
| CRD | Cysteine-rich domain |
| Crm | Cytokine response modifier |
| C-terminal | Carboxy-terminal |
| DD | Death domain |
| ddH ₂ O | Double-distilled water |
| DMEM | Dulbecco's modified Eagle's medium |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| dNTPs | Deoxyribonucleotide triphosphates |
| DOC | Sodium deoxycholate |
| dsDNA | Double stranded DNA |
| DTT | Dithiothreitol |
| EBV | Epstein-barr virus |
| EDTA | Ethylene diamine tetra acetic acid |
| Etk | Endothelial/epithelial tyrosine kinase |

| | |
|-------|--|
| EV | Enveloped virion |
| FADD | Fas associated protein with death domain |
| FAN | Factor associated with neutral shingomyelinase |
| FBS | Foetal bovine serum |
| FRET | Fluorescence resonance energy transfer |
| g | Centrigual force (gravity) |
| GITR | Glucocorticoid-induced TNF-receptor related protein ligand |
| HEK | Human embryonic kidney |
| HIV | Human immunodeficiency virus |
| HVEM | Herpes virus entry mediator |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IKK | I κ B kinase |
| IL | Interleukin |
| IRAK | Interleukin 1 receptor-associated kinase |
| IV | Immature virion |
| JNK | c-Jun N-terminal kinase |
| Kb | Kilobases |
| kDa | Kilodaltons |
| KSHV | Kaposi-s sarcoma-associated herpes virus |
| LB | Luria Broth |
| LPS | Lipopolysaccharide |
| M | Molar |
| MADD | MAPK ackivating death domain |
| MAPK | Mitogen activated protein kinase |
| MHC | Major Histocompatibility Complex |
| MPV | Monkeypox virus |
| mRNA | Messenger RNA |
| MTT | 3-(4,5 dimthylthiazolyl-2)-2,5-diphenyl tetrazolium |
| MV | Mature virion |
| MyD88 | Myeloid differentiation primary-response protein-88 |

| | |
|------------|---|
| Myx | Myxoma virus |
| NA3A | Non capsid protein 3A |
| NBT | Nitro blue tetrazolium |
| NEMO | NFκB essential modulator |
| NFκB | Nuclear factor κB |
| ng | Nanograms |
| nm | Nanometre |
| N-terminal | Amino-terminal |
| °C | Degrees Celsius |
| OGTR | Office of the Gene Technology Regulator |
| orf | Open reading frame |
| PAGE | Polyacrylamide gel electrophoresis |
| PAMPs | Pathogen associated molecular patterns |
| PBR | Poxvirus Bioinformatics Resource Center |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PLAD | Pre-ligand association domain |
| PTPC | Permeability transition pore complex |
| RA | Rheumatoid arthritis |
| RANK | Receptor activator nuclear factor κB |
| RIP | Receptor-interacting kinase |
| RNA | Ribonucleic acid |
| rpm | Revolutions per minute |
| RT-PCR | Reverse transcriptase polymerase chain reaction |
| SDS | Sodium dodecyl sulphate |
| SECRET | Smallpox virus-encoded chemokines receptor |
| SODD | Silencer of death domain |
| TAB | TAK1 binding protein |
| TACE | TNFα converting enzyme |
| TACI | Transmembrane activator of CAML interactor |
| TAK1 | Growth factor-β-activated kinase 1 |

| | |
|--------------|---|
| TBE | Tris borate buffer with EDTA |
| TIRs | Terminal inverted repeats |
| TLR | Toll-like receptor |
| TNFR | TNF receptor |
| TNFR-SF | TNFR superfamily |
| TNF-SF | TNF-superfamily |
| TNF α | Tumour necrosis factor- α |
| TRADD | TNF receptor associating death domain |
| TRAF | TNF receptor-associated factor |
| TRAIL | TNF α -related apoptosis-inducing ligand |
| TWEAK | TNF α -related weak inducer of apoptosis |
| VAC | Vaccinia virus |
| VAR | Variola virus |
| VDAC | Voltage dependent anion channel |
| VEGI | Vascular endothelial cell-growth inhibitor |
| Vpr | Viral protein R |
| WHO | World Health Organisation |
| YFP | Yellow fluorscent protein |

ABSTRACT

Tumour necrosis factor- α (TNF α) is a pleiotropic cytokine that plays a critical role in cellular response to virus infection. Virtually all poxviruses encode genes that are homologous to human tumour necrosis factor receptors (TNFRs). The viral “T2” TNFR proteins are well characterized from *Leporipox* viruses, myxoma (Myx) and Shope fibroma virus. MyxT2 has previously been shown to bind to and inhibit rabbit TNF α in a species-specific manner and, more recently, has been shown to bind to human cellular TNFRs and inhibit TNFR1-induced cell death in a non species-specific manner. In contrast, the human-tropic *Orthopoxviruses* TNFR proteins have been poorly characterised, since variola virus (VAR) existed before molecular virology capabilities and the monkeypox virus (MPV) is restricted for research. This study sought to characterize the TNFR proteins, VARG4R and MPVJ2R, encoded by strict species-specific variola virus and the broad host range monkeypox virus, and compare them to the well characterized MyxT2 protein.

With WHO Smallpox Committee approval, codon optimised cDNAs for VARG4R and MPVJ2R were constructed and these proteins were expressed as C-terminal myc-His-tagged fusion proteins by transient transfection in HEK293T cells. Both VARG4R and MPVJ2R are expressed and detectable in cell lysates and culture supernatants, exactly as occurs for MyxT2. However, while MyxT2 is both a dimer and a monomer, VARG4R is predominantly a dimer and MPVJ2R is exclusively a monomer. Secreted VARG4R and MPVJ2R are heavily glycosylated consistent with their numerous N-linked glycosylation sites. In TNF α neutralization L929 cytotoxicity assays, VARG4R inhibits rabbit, mouse, human and rhesus macaque TNF α . Although monkeypox virus has an extremely broad host range, surprisingly MPVJ2R has no TNF α inhibitory activity against rabbit, human, or rhesus macaque TNF α . Consistent with MyxT2, VARG4R and MPVJ2R inhibit TNFR1-induced cell death. It has previously been demonstrated that the viral pre-ligand association domain (PLAD) is essential for MyxT2 inhibition of TNFR1-induced cell death. Interestingly, MyxT2 PLAD, VARG4R PLAD and MPVJ2R

PLAD proteins also inhibit TNFR1-induced cell death, confirming the critical role of the viral PLAD domain in the function of these viral TNFR proteins.

Collectively these data suggest that, like MyxT2, secreted VARG4R protein acts as a functional TNF α -inhibitory factor during variola infection, but poxviruses with a broad host range, such as monkeypox, use other non species-specific mechanisms for host immune evasion. Overall, this study expands on our limited knowledge of variola and monkeypox viruses' mechanisms of immune evasion and further confirms the pivotal role of the viral PLAD in viral inhibition of TNF α -TNFR signaling.